Refine Search

Search Results -

Terms	Documents
L10 and (screening method and methotrexate)	1

Database:
- US Pre-Grant Publication Full-Text Database
- US Patents Full-Text Database
- US OCR Full-Text Database
- EPO Abstracts Database
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- IBM Technical Disclosure Bulletins

Search: L11

Refine Search | Recall Text | Clear | Interrupt

Search History

DATE: Thursday, August 31, 2006 **Purge Queries** Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=PGPB; PLUR=YES; OP=OR			
L11	L10 and (screening method and methotrexate)	1	L11
L10	20020004202	1	L10
L9	L8 and l4	7	L9
L8	L7 and (expression)	11670	L8
L7	L6 and (cell and reporter gene)	11973	L7
L6	L5 and ligand	12751	L6
L5	(protein target) and methotrexate	18501	L5
L4	cornish.in.	67	L4
L3	L1 and (protein target)	1	L3
L2	L1 and (analog of methotrexate)	1	L2
L1	20040106154	1	L1

END OF SEARCH HISTORY

Refine Search

Search Results -

Terms	Documents
L1 and (protein target)	1

Database:
- US Pre-Grant Publication Full-Text Database
- US Patents Full-Text Database
- US OCR Full-Text Database
- EPO Abstracts Database
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Search: L3

Refine Search | Recall Text | Clear | Interrupt

Search History

DATE: Thursday, August 31, 2006 Purge Queries | Printable Copy | Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=PGPB; PLUR=YES; OP=OR			
L3	L1 and (protein target)	1	L3
L2	L1 and (analog of methotrexate)	1	L2
L1	20040106154	1	L1

END OF SEARCH HISTORY

Refine Search

Search Results -

Terms	Documents
L8 and L4	7

Database:
US Pre-Grant Publication Full-Text Database
US Patents Full-Text Database
US OCR Full-Text Database
EPO Abstracts Database
JPO Abstracts Database
Derwent World Patents Index
IBM Technical Disclosure Bulletins

Search: L9

Refine Search

Recall Text | Clear | Interrupt

Search History

DATE: Thursday, August 31, 2006 Purge Queries Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=PGPB; PLUR=YES; OP=OR			
L9	L8 and l4	7	L9
L8	L7 and (expression)	11670	L8
L7	L6 and (cell and reporter gene)	11973	L7
L6	L5 and ligand	12751	L6
L5	(protein target) and methotrexate	18501	L5
L4	cornish.in.	67	L4
L3	L1 and (protein target)	1	L3
L2	L1 and (analog of methotrexate)	1	L2
L1	20040106154	1	L1

END OF SEARCH HISTORY

Hit List

Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

First Hit

Search Results - Record(s) 1 through 7 of 7 returned.

☐ 1. Document ID: US 20050221402 A1

L9: Entry 1 of 7 File: PGPB Oct 6, 2005

PGPUB-DOCUMENT-NUMBER: 20050221402
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20050221402 A1

TITLE: Bacterial small-molecule three-hybrid system

PUBLICATION-DATE: October 6, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Althoff, Eric A	New York	NY	US
Cornish, Virginia W	New York	NY	US

US-CL-CURRENT: 435/7.32; 435/252.3, 435/471

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

☐ 2. Document ID: US 20040106154 A1

L9: Entry 2 of 7 File: PGPB Jun 3, 2004

PGPUB-DOCUMENT-NUMBER: 20040106154
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040106154 A1

TITLE: In vivo screen using chemical inducers of dimerization

PUBLICATION-DATE: June 3, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Cornish, Virginia W.	New York	NY	US

US-CL-CURRENT: 435/7.1; 530/300

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

☐ 3. Document ID: US 20030203471 A1

L9: Entry 3 of 7 File: PGPB Oct 30, 2003

PGPUB-DOCUMENT-NUMBER: 20030203471
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030203471 A1

TITLE: Bacterial small-molecule three-hybrid system

PUBLICATION-DATE: October 30, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Althoff, Eric A.	New York	NY	US
Cornish, Virginia W.	New York	NY	US

US-CL-CURRENT: 435/252.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

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☐ 4. Document ID: US 20030138785 A1

L9: Entry 4 of 7 File: PGPB Jul 24, 2003

PGPUB-DOCUMENT-NUMBER: 20030138785
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030138785 A1

TITLE: In vivo protein screen based on enzyme-assisted chemically induced dimerization ("CID")

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Kopytek, Stephan	New York	NY	US
Cornish, Virginia	New York	NY	US

US-CL-CURRENT: 435/6; 435/455, 435/7.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

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☐ 5. Document ID: US 20020168737 A1

L9: Entry 5 of 7 File: PGPB Nov 14, 2002

PGPUB-DOCUMENT-NUMBER: 20020168737
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020168737 A1

TITLE: Binding and catalysis screen for high throughput determination of protein function using chemical inducers of dimerization

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Cornish, Virginia W.	New York	NY	US

US-CL-CURRENT: 435/188.5; 435/231

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

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☐ 6. Document ID: US 20020168685 A1

L9: Entry 6 of 7 File: PGPB Nov 14, 2002

PGPUB-DOCUMENT-NUMBER: 20020168685
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020168685 A1

TITLE: Covalent chemical inducers of protein dimerization and their uses in high throughput binding screens

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Cornish, Virginia W.	New York	NY	US

US-CL-CURRENT: 435/7.1; 536/27.13, 536/28.53, 540/222, 544/259

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

☐ 7. Document ID: US 20020004202 A1

L9: Entry 7 of 7 File: PGPB Jan 10, 2002

PGPUB-DOCUMENT-NUMBER: 20020004202
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020004202 A1

TITLE: In vivo screen using chemical inducers of dimerization

PUBLICATION-DATE: January 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Cornish, Virginia W.	New York	NY	US

US-CL-CURRENT: 435/6; 540/109

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

Terms	Documents
L8 and L4	7

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 NEWS  3  FEB 27  New STN AnaVist pricing effective March 1, 2006
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 NEWS  5  MAY 10  CA/CAplus enhanced with 1900-1906 U.S. patent records
 NEWS  6  MAY 11  KOREAPAT updates resume
 NEWS  7  MAY 19  Derwent World Patents Index to be reloaded and enhanced
 NEWS  8  MAY 30  IPC 8 Rolled-up Core codes added to CA/CAplus and
                  USPATFULL/USPAT2
 NEWS  9  MAY 30  The F-Term thesaurus is now available in CA/CAplus
 NEWS 10  JUN 02  The first reclassification of IPC codes now complete in
                  INPADOC
 NEWS 11  JUN 26  TULSA/TULSA2 reloaded and enhanced with new search and
                  and display fields
 NEWS 12  JUN 28  Price changes in full-text patent databases EPFULL and PCTFULL
 NEWS 13  JUl 11  CHEMSAFE reloaded and enhanced
 NEWS 14  JUl 14  FSTA enhanced with Japanese patents
 NEWS 15  JUl 19  Coverage of Research Disclosure reinstated in DWPI
 NEWS 16  AUG 09  INSPEC enhanced with 1898-1968 archive
 NEWS 17  AUG 28  ADISCTI Reloaded and Enhanced
 NEWS 18  AUG 30  CA(SM)/CAplus(SM) Austrian patent law changes

 NEWS EXPRESS  JUNE 30 CURRENT WINDOWS VERSION IS V8.01b, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 JUNE 2006.

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=> s (3-hybrid screen) and (methotrexate)
L1 0 (3-HYBRID SCREEN) AND (METHOTREXATE)

=> s (Y3H or three hybrid) and (methotrexate)
L2 30 (Y3H OR THREE HYBRID) AND (METHOTREXATE)

=> s l2 and (fusion protein)
L3 7 L2 AND (FUSION PROTEIN)

=> s l2 and (reporter gene)
L4 14 L2 AND (REPORTER GENE)

=> s l3 and l4
L5 4 L3 AND L4

=> d l5 ti abs ibib tot

L5 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Bacterial small-molecule three-hybrid system based on the interaction of heterodimeric ligand-receptor interaction and use thereof for high-throughput drug screening
AB A transgenic bacterial cell comprising (a) a dimeric small mol. which comprises a first moiety known to bind a first receptor domain covalently linked to a second moiety known to bind a second receptor domain; (b) nucleotide sequences which upon transcription encode (i) a first fusion protein comprising the first receptor domain, and (ii) a second fusion protein comprising the second receptor domain; and (c) a reporter gene wherein expression of the reporter gene is conditioned on the proximity of the first fusion protein to the second fusion protein. The cell is also adapted for use in a method for identifying a mol. that binds to a known target in a bacterial cell from a pool of candidate mols., and a method for identifying an unknown target receptor to which a mol. is capable of binding in a bacterial cell. Also described are compds. and kits for carrying out the methods, in particular, the synthesis of the Mtx-SLF heterodimer. In particular embodiments, the method is exemplified by using a small mol. heterodimeric Mtx-SLF (methotrexate-SLF(a synthetic analog of FK506)) to bridge the λcI DNA-binding domain, which is fused to FK506 receptor FKBP12 (FK506-binding protein 12), and the activation domain - αNTD (the N-terminal domain of the α-subunit of RNA polymerase), which is fused to methotrexate receptor DHFR (dihydrofolate reductase). The interaction of λcI-FKBP12 and αNTD-DHFR fusion protein leads to the transcription activation of a lacZ reporter gene, in

which the λcI binding site is placed upstream of lacZ promoter. Thus, upon addition of the small mol. heterodimer Mtx-SLF, the λcI-FKBP12 and αNTD-DHFR fusion protein are dimerized, which then drives the lacZ transcription. This bacterial small mol. three-hybrid system is useful for high-throughput screening for small mol. drugs and drug-interacting protein targets.

ACCESSION NUMBER: 2005:1078092 HCAPLUS
DOCUMENT NUMBER: 143:361162
TITLE: Bacterial small-molecule three-hybrid system based on the interaction of heterodimeric ligand-receptor interaction and use thereof for high-throughput drug screening
INVENTOR(S): Althoff, Eric A.; Cornish, Virginia W.
PATENT ASSIGNEE(S): Trustees of Columbia University In the City of New York, USA
SOURCE: U.S. Pat. Appl. Publ., 28 pp., Cont.-in-part of U.S. Ser. No. 132,039.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005221402	A1	20051006	US 2005-512497	20050523
US 2003203471	A1	20031030	US 2002-132039	20020424
US 7083918	B2	20060801		
WO 2004042345	A2	20040521	WO 2003-US12612	20030424
WO 2004042345	A3	20040923		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2002-132039 A2 20020424
WO 2003-US12612 W 20030424
OTHER SOURCE(S): MARPAT 143:361162

L5 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Three hybrid assay system for isolating ligand-binding polypeptides and for isolating small mol. ligands
AB The invention provides compns. and methods for isolating ligand-binding polypeptides for a user-specified ligand, and for isolating small mol. ligands for a user-specified target polypeptide using an improved class of hybrid ligand compds. Preparation of compds., e.g a methotrexate moiety linked by a polyethylene gycol moiety to dexamethasone, is described.
ACCESSION NUMBER: 2004:182368 HCAPLUS
DOCUMENT NUMBER: 140:229401
TITLE: Three hybrid assay system for isolating ligand-binding polypeptides and for isolating small mol. ligands
INVENTOR(S): Come, Jon H.; Becker, Frank; Kley, Nikolai A.; Reichel, Christoph
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 238 pp., Cont.-in-part of U.S.

Ser. No. 91,177.
CODEN: USXXCO

bad date

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 6
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
US 2004043388	A1	20040304	US 2002-234985		20020903
US 2003165873	A1	20030904	US 2002-91177		20020304
US 2004266854	A1	20041230	US 2004-820453		20040407
PRIORITY APPLN. INFO.:			US 2001-272932P	P	20010302
			US 2001-278233P	P	20010323
			US 2001-329437P	P	20011015
			US 2002-91177	A2	20020304
			US 2001-336962P	P	20011203
			WO 2002-US6677	A2	20020304
			US 2002-234985	A2	20020903
			WO 2002-US33052	A2	20021015
			US 2003-460921P	P	20030407
			US 2003-531872P	P	20031223

L5 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2006 ACS on STN

TI Bacterial small-mol. three-hybrid system comprising dimeric Mtx-SLF ligand that bridges λcI and NTD fusion proteins for detecting protein-small molecule interactions

AB The present invention provides a transgenic bacterial cell comprising (a) a dimeric small mol. which comprises a first moiety known to bind a first receptor domain covalently linked to a second moiety known to bind a second receptor domain; (b) nucleotide sequences which upon transcription encode (i) a first fusion protein comprising the first receptor domain, and (ii) a second fusion protein comprising the second receptor domain; and (c) a reporter gene wherein expression of the reporter gene is conditioned on the proximity of the first fusion protein to the second fusion protein. The cell is also adapted for use in a method for identifying a mol. that binds to a known target in a bacterial cell from a pool of candidate mols., and a method for identifying an unknown target receptor to which a mol. is capable of binding in a bacterial cell. Also described are compds. and kits for carrying out the methods. The examples describe the synthetic preparation of a heterodimer of methotrexate and a synthetic analog of FK507 (SLF), referred to as Mtx-SLF. Mtx-SLF was used to dimerize a λcI-FK506 binding protein 12 protein chimera and an αNTD-dihydrofolate reductase protein chimera.

ACCESSION NUMBER: 2003:855546 HCAPLUS
DOCUMENT NUMBER: 139:346749
TITLE: Bacterial small-mol. three-hybrid system comprising dimeric Mtx-SLF ligand that bridges λcI and NTD fusion proteins for detecting protein-small molecule interactions
INVENTOR(S): Althoff, Eric A.; Cornish, Virginia W.
PATENT ASSIGNEE(S): The Trustees of Columbia University In the City of New York, USA
SOURCE: U.S. Pat. Appl. Publ., 28 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

US 2003203471	A1	20031030	US 2002-132039	20020424
US 7083918	B2	20060801		
WO 2004042345	A2	20040521	WO 2003-US12612	20030424
WO 2004042345	A3	20040923		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2003299463	A1	20040607	AU 2003-299463	20030424
US 2005221402	A1	20051006	US 2005-512497	20050523
PRIORITY APPLN. INFO.:			US 2002-132039	A2 20020424
			WO 2003-US12612	W 20030424

OTHER SOURCE(S): MARPAT 139:346749

L5 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2006 ACS on STN

TI A bacterial small-molecule three-hybrid system

AB The authors report the first robust bacterial RNA polymerase small mol. three-hybrid system. This system is based on the interaction between the small mol. methotrexate and a synthetic analog of FK506 (SLF) and their protein receptors, dihydrofolate reductase and FK506-binding protein 12 (FKBP12). In this assay, the binding site for the DNA-binding protein λcI is placed upstream of the promoter for a lacZ reporter gene. λCI is fused to FKBP12 and the N-terminal domain of the α-subunit of RNA polymerase (αNTD) is fused to DHFR. Thus, upon addition of the small mol. heterodimer Mtx-SLF, the λcI-FKBP12 and αNTD-DHFR fusion protein are dimerized, thus activating transcription of the lacZ gene. Synthesis of the Mtx-SLF heterodimer is described. The levels of small mol. induced transcription activation were quantified using liquid lacZ assays. The levels of transcriptional activation depend on the concentration of Mtx-SLF in the bacterial three -hybrid system. The bacterial small mol. three-hybrid system described here should provide a platform for high-throughput assays based on protein-small mol. interactions.

ACCESSION NUMBER: 2002:548931 HCAPLUS
DOCUMENT NUMBER: 137:305334
TITLE: A bacterial small-molecule three-hybrid system
AUTHOR(S): Althoff, Eric A.; Cornish, Virginia W.
CORPORATE SOURCE: Department of Chemistry, Columbia University, New York, NY, 10027, USA
SOURCE: Angewandte Chemie, International Edition (2002), 41(13), 2327-2330
CODEN: ACIEF5; ISSN: 1433-7851
PUBLISHER: Wiley-VCH Verlag GmbH
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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(FILE 'HOME' ENTERED AT 11:29:15 ON 31 AUG 2006)

FILE 'MEDLINE, BIOSIS, WPIDS, BIOTECHDS, HCAPLUS' ENTERED AT 11:33:17 ON

31 AUG 2006
L1 0 S (3-HYBRID SCREEN) AND (METHOTREXATE)
L2 30 S (Y3H OR THREE HYBRID) AND (METHOTREXATE)
L3 7 S L2 AND (FUSION PROTEIN)
L4 14 S L2 AND (REPORTER GENE)
L5 4 S L3 AND L4

=> d l3 ti abs ibib tot

L3 ANSWER 1 OF 7 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
TI Induced protein dimerization in vivo through covalent labeling.
ACCESSION NUMBER: 2004:108496 BIOSIS
DOCUMENT NUMBER: PREV200400110447
TITLE: Induced protein dimerization in vivo through covalent labeling.
AUTHOR(S): Gendreizig, Susanne; Kindermann, Maik; Johnsson, Kai [Reprint Author]
CORPORATE SOURCE: Institute of Molecular and Biological Chemistry, Ecole Polytechnique Federale de Lausanne (EPFL), CH-1015, Lausanne, Switzerland
kai.johnsson@epfl.ch
SOURCE: Journal of the American Chemical Society, (December 10 2003) Vol. 125, No. 49, pp. 14970-14971. print.
ISSN: 0002-7863 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 25 Feb 2004
Last Updated on STN: 25 Feb 2004

L3 ANSWER 2 OF 7 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Bacterial small-molecule three-hybrid system based on the interaction of heterodimeric ligand-receptor interaction and use thereof for high-throughput drug screening
AB A transgenic bacterial cell comprising (a) a dimeric small mol. which comprises a first moiety known to bind a first receptor domain covalently linked to a second moiety known to bind a second receptor domain; (b) nucleotide sequences which upon transcription encode (i) a first fusion protein comprising the first receptor domain, and (ii) a second fusion protein comprising the second receptor domain; and (c) a reporter gene wherein expression of the reporter gene is conditioned on the proximity of the first fusion protein to the second fusion protein. The cell is also adapted for use in a method for identifying a mol. that binds to a known target in a bacterial cell from a pool of candidate mols., and a method for identifying an unknown target receptor to which a mol. is capable of binding in a bacterial cell. Also described are compds. and kits for carrying out the methods, in particular, the synthesis of the Mtx-SLF heterodimer. In particular embodiments, the method is exemplified by using a small mol. heterodimeric Mtx-SLF (methotrexate-SLF(a synthetic analog of FK506)) to bridge the λcI DNA-binding domain, which is fused to FK506 receptor FKBP12 (FK506-binding protein 12), and the activation domain - αNTD (the N-terminal domain of the α-subunit of RNA polymerase), which is fused to methotrexate receptor DHFR (dihydrofolate reductase). The interaction of λcI-FKBP12 and αNTD-DHFR fusion protein leads to the transcription activation of a lacZ reporter gene, in which the λcI binding site is placed upstream of lacZ promoter. Thus, upon addition of the small mol. heterodimer Mtx-SLF, the λcI-FKBP12 and αNTD-DHFR fusion protein are dimerized, which then drives the lacZ transcription. This bacterial small mol. three-hybrid system is useful for high-throughput screening for small mol. drugs and drug-interacting protein targets.
ACCESSION NUMBER: 2005:1078092 HCAPLUS

DOCUMENT NUMBER: 143:361162
TITLE: Bacterial small-molecule three-hybrid system based on the interaction of heterodimeric ligand-receptor interaction and use thereof for high-throughput drug screening
INVENTOR(S): Althoff, Eric A.; Cornish, Virginia W.
PATENT ASSIGNEE(S): Trustees of Columbia University In the City of New York, USA
SOURCE: U.S. Pat. Appl. Publ., 28 pp., Cont.-in-part of U.S. Ser. No. 132,039.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005221402	A1	20051006	US 2005-512497	20050523
US 2003203471	A1	20031030	US 2002-132039	20020424
US 7083918	B2	20060801		
WO 2004042345	A2	20040521	WO 2003-US12612	20030424
WO 2004042345	A3	20040923		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2002-132039 A2 20020424
WO 2003-US12612 W 20030424
OTHER SOURCE(S): MARPAT 143:361162

L3 ANSWER 3 OF 7 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Three hybrid assay system for isolating ligand-binding polypeptides and for isolating small mol. ligands
AB The invention provides compns. and methods for isolating ligand-binding polypeptides for a user-specified ligand, and for isolating small mol. ligands for a user-specified target polypeptide using an improved class of hybrid ligand compds. Preparation of compds., e.g a methotrexate moiety linked by a polyethylene gycol moiety to dexamethasone, is described.
ACCESSION NUMBER: 2004:182368 HCAPLUS
DOCUMENT NUMBER: 140:229401
TITLE: Three hybrid assay system for isolating ligand-binding polypeptides and for isolating small mol. ligands
INVENTOR(S): Come, Jon H.; Becker, Frank; Kley, Nikolai A.; Reichel, Christoph
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 238 pp., Cont.-in-part of U.S. Ser. No. 91,177.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 6
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

US 2004043388	A1	20040304	US 2002-234985		20020903
US 2003165873	A1	20030904	US 2002-91177		20020304
US 2004266854	A1	20041230	US 2004-820453		20040407
PRIORITY APPLN. INFO.:			US 2001-272932P	P	20010302
			US 2001-278233P	P	20010323
			US 2001-329437P	P	20011015
			US 2002-91177	A2	20020304
			US 2001-336962P	P	20011203
			WO 2002-US6677	A2	20020304
			US 2002-234985	A2	20020903
			WO 2002-US33052	A2	20021015
			US 2003-460921P	P	20030407
			US 2003-531872P	P	20031223

L3 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2006 ACS on STN

TI Bacterial small-mol. three-hybrid system comprising dimeric Mtx-SLF ligand that bridges λcI and NTD fusion proteins for detecting protein-small molecule interactions

AB The present invention provides a transgenic bacterial cell comprising (a) a dimeric small mol. which comprises a first moiety known to bind a first receptor domain covalently linked to a second moiety known to bind a second receptor domain; (b) nucleotide sequences which upon transcription encode (i) a first fusion protein comprising the first receptor domain, and (ii) a second fusion protein comprising the second receptor domain; and (c) a reporter gene wherein expression of the reporter gene is conditioned on the proximity of the first fusion protein to the second fusion protein. The cell is also adapted for use in a method for identifying a mol. that binds to a known target in a bacterial cell from a pool of candidate mols., and a method for identifying an unknown target receptor to which a mol. is capable of binding in a bacterial cell. Also described are compds. and kits for carrying out the methods. The examples describe the synthetic preparation of a heterodimer of methotrexate and a synthetic analog of FK507 (SLF), referred to as Mtx-SLF. Mtx-SLF was used to dimerize a λcI-FK506 binding protein 12 protein chimera and an αNTD-dihydrofolate reductase protein chimera.

ACCESSION NUMBER: 2003:855546 HCAPLUS
DOCUMENT NUMBER: 139:346749
TITLE: Bacterial small-mol. three-hybrid system comprising dimeric Mtx-SLF ligand that bridges λcI and NTD fusion proteins for detecting protein-small molecule interactions
INVENTOR(S): Althoff, Eric A.; Cornish, Virginia W.
PATENT ASSIGNEE(S): The Trustees of Columbia University In the City of New York, USA
SOURCE: U.S. Pat. Appl. Publ., 28 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003203471	A1	20031030	US 2002-132039	20020424
US 7083918	B2	20060801		
WO 2004042345	A2	20040521	WO 2003-US12612	20030424
WO 2004042345	A3	20040923		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM,

PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT,
TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,
FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR,
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2003299463	A1	20040607	AU 2003-299463	20030424
US 2005221402	A1	20051006	US 2005-512497	20050523
PRIORITY APPLN. INFO.:			US 2002-132039	A2 20020424
			WO 2003-US12612	W 20030424

OTHER SOURCE(S): MARPAT 139:346749

L3 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2006 ACS on STN
TI A bacterial small-molecule three-hybrid system
AB The authors report the first robust bacterial RNA polymerase small mol. three-hybrid system. This system is based on the interaction between the small mol. methotrexate and a synthetic analog of FK506 (SLF) and their protein receptors, dihydrofolate reductase and FK506-binding protein 12 (FKBP12). In this assay, the binding site for the DNA-binding protein λcI is placed upstream of the promoter for a lacZ reporter gene. λCI is fused to FKBP12 and the N-terminal domain of the α-subunit of RNA polymerase (αNTD) is fused to DHFR. Thus, upon addition of the small mol. heterodimer Mtx-SLF, the λcI-FKBP12 and αNTD-DHFR fusion protein are dimerized, thus activating transcription of the lacZ gene. Synthesis of the Mtx-SLF heterodimer is described. The levels of small mol. induced transcription activation were quantified using liquid lacZ assays. The levels of transcriptional activation depend on the concentration of Mtx-SLF in the bacterial three-hybrid system. The bacterial small mol. three-hybrid system described here should provide a platform for high-throughput assays based on protein-small mol. interactions.

ACCESSION NUMBER:	2002:548931 HCAPLUS
DOCUMENT NUMBER:	137:305334
TITLE:	A bacterial small-molecule three-hybrid system
AUTHOR(S):	Althoff, Eric A.; Cornish, Virginia W.
CORPORATE SOURCE:	Department of Chemistry, Columbia University, New York, NY, 10027, USA
SOURCE:	Angewandte Chemie, International Edition (2002), 41(13), 2327-2330 CODEN: ACIEF5; ISSN: 1433-7851
PUBLISHER:	Wiley-VCH Verlag GmbH
DOCUMENT TYPE:	Journal
LANGUAGE:	English
REFERENCE COUNT:	27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Characterization of the Dexamethasone-Methotrexate yeast three-hybrid system
AB A novel Chemical Inducer of Dimerization (CID), Dexamethasone-Methotrexate (Dex-Mtx), has been shown to effectively dimerize its receptor proteins in a yeast three-hybrid assay, where DHFR, the Mtx receptor, was bound to a DNA binding domain, and GR, the Dex receptor, was bound to a transcription activation domain. In order to characterize the Dex-Mtx system, systematic modifications were introduced to its different components, and their effect on dimerization efficiency was observed Several Dex-Mtx mols. were synthesized using aliphatic linkers of varying lengths, DHFR from a bacterial and mammalian source was used, the orientation of the fusion protein domains was reversed, and small peptide linkers were added between the domains. Beta-galactosidase activity was used as the reporter for dimerization

efficiency.
ACCESSION NUMBER: 2002:187333 HCAPLUS
TITLE: Characterization of the Dexamethasone-Methotrexate yeast three-hybrid system
AUTHOR(S): Abida, Wassim M.; Carter, Brian T.; Althoff, Eric A.; Lin, Hening; Cornish, Virginia W.
CORPORATE SOURCE: Department of Chemistry, Columbia University, New York, NY, 10027, USA
SOURCE: Abstracts of Papers, 223rd ACS National Meeting, Orlando, FL, United States, April 7-11, 2002 (2002), CHED-702. American Chemical Society: Washington, D. C.
CODEN: 69CKQP
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English

L3 ANSWER 7 OF 7 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Dexamethasone-Methotrexate: An Efficient Chemical Inducer of Protein Dimerization In Vivo
AB A heterodimeric dexamethasone-methotrexate compound (Dex-Mtx) was prepared that can dimerize proteins efficiently in vivo. A yeast three-hybrid system and a standard β-galactosidase assay were used to show that Dex-Mtx (prepared in 8 steps in 2% overall yield) can activate lacZ transcription in vivo.
ACCESSION NUMBER: 2000:238920 HCAPLUS
DOCUMENT NUMBER: 133:86413
TITLE: Dexamethasone-Methotrexate: An Efficient Chemical Inducer of Protein Dimerization In Vivo
AUTHOR(S): Lin, Hening; Abida, Wassim M.; Sauer, Robert T.; Cornish, Virginia W.
CORPORATE SOURCE: Department of Chemistry, Columbia University, New York, NY, 10027, USA
SOURCE: Journal of the American Chemical Society (2000), 122(17), 4247-4248
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 11:29:15 ON 31 AUG 2006)

FILE 'MEDLINE, BIOSIS, WPIDS, BIOTECHDS, HCAPLUS' ENTERED AT 11:33:17 ON 31 AUG 2006
L1 0 S (3-HYBRID SCREEN) AND (METHOTREXATE)
L2 30 S (Y3H OR THREE HYBRID) AND (METHOTREXATE)
L3 7 S L2 AND (FUSION PROTEIN)
L4 14 S L2 AND (REPORTER GENE)
L5 4 S L3 AND L4

=> d l4 ti abs ibib tot

L4 ANSWER 1 OF 14 MEDLINE on STN
TI Correlation between ligand-receptor affinity and the transcription readout in a yeast three-hybrid system.
AB The yeast two-hybrid assay has proven to be a powerful method to detect protein-protein interactions as well as to derive genome-wide protein interaction maps. More recently, three-hybrid assays

have emerged as a means to detect both protein-RNA and protein-small molecule interactions. Despite the routine use of the two-hybrid assay and the potential of three-hybrid systems, there has been little quantitative characterization to understand how the strength of the protein interaction correlates with transcription activation. It is not known if the additional interaction in three-hybrid systems compromises the sensitivity of the system. Thus, here, we set out to determine the K(D) cutoff of a small molecule three-hybrid system and to determine if there is a correlation between the K(D) and the levels of transcription activation. A series of mutations to FK506-binding protein 12 (FKBP12) were designed to vary the affinity of this protein for the small molecule synthetic ligand for FK506-binding protein 12 (SLF). These FKBP12 variants were overexpressed and purified, and their K(D)'s for SLF were measured using a fluorescence polarization assay. Then the levels of transcription activation in a Mtx-DHFR yeast three-hybrid system were determined for these variants using a lacZ reporter gene. The K(D) cutoff of the Mtx yeast three-hybrid system is found to be ca. 50 nM. Further, the levels of transcription activation correlate with the strength of the binding interaction, though the dynamic range is only 1 order of magnitude. These results establish that the three-hybrid assay has the requisite sensitivity for drug discovery. However, the small dynamic range highlights a limitation to equilibrium-based assays for discriminating interactions based on affinity.

ACCESSION NUMBER: 2004397997 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15301533
TITLE: Correlation between ligand-receptor affinity and the transcription readout in a yeast three-hybrid system.
AUTHOR: de Felipe Karim Suwwan; Carter Brian T; Althoff Eric A; Cornish Virginia W
CORPORATE SOURCE: Integrated Program in Cellular, Molecular, and Biophysical Studies, Columbia University, New York, New York 10027, USA.
CONTRACT NUMBER: R01-GM62867 (NIGMS)
SOURCE: Biochemistry, (2004 Aug 17) Vol. 43, No. 32, pp. 10353-63. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200409
ENTRY DATE: Entered STN: 11 Aug 2004
Last Updated on STN: 15 Sep 2004
Entered Medline: 14 Sep 2004

L4 ANSWER 2 OF 14 MEDLINE on STN

TI Correlation between catalytic efficiency and the transcription read-out in chemical complementation: a general assay for enzyme catalysis.

AB High-throughput assays for enzyme catalysis that can be applied to a broad range of chemical reactions are key to advances in directed evolution and proteomics. Recently, we reported such a general assay, chemical complementation, which links enzyme catalysis to reporter gene transcription in vivo using the yeast three-hybrid assay. In this proof-of-principle experiment, it was shown that a wild-type beta-lactamase enzyme could be isolated from a pool of inactive mutants using a lacZ screen. Ideally, however, such an assay should be able to distinguish enzymes based on their catalytic activity. Thus, here, we set out to determine if the catalytic efficiency of an enzyme variant does in fact correlate with its level of transcription activation in the chemical complementation assay. First, the reaction mechanism for the cleavage of the beta-lactam substrate used in the

chemical complementation proof-of-principle experiment was determined. Then a series of beta-lactamase variants was designed to span several orders of magnitude in k(cat)/K(m). The activity of each variant was determined both in vitro using purified enzyme and in vivo in the chemical complementation transcription assay. Beta-lactamase variants spanning three-orders of magnitude in k(cat)/K(m) could be distinguished in the assay, and the catalytic efficiency of each variant correlated with its level of transcription activation in vivo. These results establish the suitability of chemical complementation for the directed evolution of enzymes with improvements in catalytic activity and for profiling the relative substrate specificities of a group of enzymes in proteomics applications.

ACCESSION NUMBER: 2004142974 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15035627
TITLE: Correlation between catalytic efficiency and the transcription read-out in chemical complementation: a general assay for enzyme catalysis.
AUTHOR: Sengupta Debleena; Lin Hening; Goldberg Shalom D; Mahal Jacqueline J; Cornish Virginia W
CORPORATE SOURCE: Department of Chemistry, Columbia University, New York, New York 10027, USA.
CONTRACT NUMBER: R01-GM062867 (NIGMS)
SOURCE: Biochemistry, (2004 Mar 30) Vol. 43, No. 12, pp. 3570-81. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200407
ENTRY DATE: Entered STN: 24 Mar 2004
Last Updated on STN: 22 Jul 2004
Entered Medline: 21 Jul 2004

L4 ANSWER 3 OF 14 MEDLINE on STN
TI Receptor-dependence of the transcription read-out in a small-molecule three-hybrid system.
AB Small-molecule three-hybrid systems show promise as an in vivo alternative to affinity chromatography for detecting small-molecule-protein interactions. While several three-hybrid systems have been reported, little has been done to characterize these systems and, in particular, to test the assumption that the protein-small-molecule interaction can be varied without disrupting the transcription read-out. Recently we reported a dexamethasone-methotrexate chemical inducer of dimerization (CID) for use in the yeast three-hybrid system, based on the well-studied ligand-receptor pairs dexamethasone (Dex)-glucocorticoid receptor (GR) and methotrexate (Mtx)-dihydrofolate reductase (DHFR). Here we describe our first efforts to characterize this system, by focusing on a comparison of the activity of a bacterial and a mammalian DHFR as a test case of the influence of the ligand-receptor pair on the transcription read-out. By using a lacZ reporter gene, the activity of several GR and DHFR protein chimeras with different orientations and linker sequences and Dex-Mtx CIDs with different chemical linkers have been compared. In addition, Western analyses and in vivo biochemical assays have been carried out to confirm the integrity of the GR and DHFR protein chimeras. The transcription read-out is found to be much more sensitive to the structure of the protein chimeras than the CID. The most surprising result is that the levels of transcription activation are consistently higher with the bacterial than the mammalian DHFR, despite the fact that both proteins bind Mtx with an inhibition constant (K(I)) in the low pM range. These results set the stage for understanding three-hybrid systems at the biochemical level so that they can be used to detect ligand-receptor pairs with a range of

structures and dissociation constants.
ACCESSION NUMBER: 2002453164 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12210990
TITLE: Receptor-dependence of the transcription read-out in a small-molecule three-hybrid system.
AUTHOR: Abida Wassim M; Carter Brian T; Althoff Eric A; Lin Hening; Cornish Virginia W
CORPORATE SOURCE: Department of Chemistry, Columbia University, New York, NY 10027, USA.
SOURCE: Chembiochem : a European journal of chemical biology, (2002 Sep 2) Vol. 3, No. 9, pp. 887-95.
Journal code: 100937360. ISSN: 1439-4227.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200307
ENTRY DATE: Entered STN: 6 Sep 2002
Last Updated on STN: 15 Jul 2003
Entered Medline: 14 Jul 2003

L4 ANSWER 4 OF 14 MEDLINE on STN
TI A GAL4-based yeast three-hybrid system for the identification of small molecule-target protein interactions.
AB We report the development of a yeast strain designed for assaying compound-protein interactions through activation of reporter gene expression. Activation of lacZ expression, driven by the GAL4 promoter, has been demonstrated for precedented compound-protein interactions between FK506 and FK506 binding protein 12 (FKBP12) and also between methotrexate (MTX) and dihydrofolate reductase (DHFR). Reporter gene expression was completely abrogated in a competitive manner by the presence of excess FK506 or MTX, respectively. In addition, a strain expressing a mutated DHFR clone with decreased binding affinity for MTX was not capable of activating reporter gene expression. While strain sensitivity is compound-dependent, the minimum compound concentration necessary to drive reporter gene expression was 20 nM for the FK506-FKBP12 interaction. The utility of this strain as a tool for identifying unknown compound-binding proteins has been demonstrated by screening a mouse cDNA library for clones that encode proteins capable of binding MTX. Four library clones of mouse DHFR were identified after screening 5 x 10(6) clones. The screen background was low and false positives were easily identified, making this yeast system particularly amenable for use in a screening context for novel compound-protein interactions.
ACCESSION NUMBER: 2002268523 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12007565
TITLE: A GAL4-based yeast three-hybrid system for the identification of small molecule-target protein interactions.
AUTHOR: Henthorn Debbie C; Jaxa-Chamiec Albert A; Meldrum Eric
CORPORATE SOURCE: Asthma Cell Biology, GlaxoSmithKline Medicines Research Centre, Gunnels Wood Road, Hertfordshire, Stevenage, UK.. dch47508@gsk.com
SOURCE: Biochemical pharmacology, (2002 May 1) Vol. 63, No. 9, pp. 1619-28.
Journal code: 0101032. ISSN: 0006-2952.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 15 May 2002
Last Updated on STN: 12 Jul 2002

Entered Medline: 10 Jul 2002

L4 ANSWER 5 OF 14 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
TI A GAL4-based yeast three-hybrid system for the identification of small molecule-target protein interactions.
AB We report the development of a yeast strain designed for assaying compound-protein interactions through activation of reporter gene expression. Activation of lacZ expression, driven by the GAL4 promoter, has been demonstrated for precedented compound-protein interactions between FK506 and FK506 binding protein 12 (FKBP12) and also between methotrexate (MTX) and dihydrofolate reductase (DHFR). Reporter gene expression was completely abrogated in a competitive manner by the presence of excess FK506 or MTX, respectively. In addition, a strain expressing a mutated DHFR clone with decreased binding affinity for MTX was not capable of activating reporter gene expression. While strain sensitivity is compound-dependent, the minimum compound concentration necessary to drive reporter gene expression was 20 nM for the FK506-FKBP12 interaction. The utility of this strain as a tool for identifying unknown compound-binding proteins has been demonstrated by screening a mouse cDNA library for clones that encode proteins capable of binding MTX. Four library clones of mouse DHFR were identified after screening 5 X 106 clones. The screen background was low and false positives were easily identified, making this yeast system particularly amenable for use in a screening context for novel compound-protein interactions.
ACCESSION NUMBER: 2002:402745 BIOSIS
DOCUMENT NUMBER: PREV200200402745
TITLE: A GAL4-based yeast three-hybrid system for the identification of small molecule-target protein interactions.
AUTHOR(S): Henthorn, Debbie C. [Reprint author]; Jaxa-Chamiec, Albert A.; Meldrum, Eric
CORPORATE SOURCE: Asthma Cell Biology, GlaxoSmithKline Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, UK
dch47508@gsk.com; em13714@gsk.com
SOURCE: Biochemical Pharmacology, (1 May, 2002) Vol. 63, No. 9, pp. 1619-1628. print.
CODEN: BCPCA6. ISSN: 0006-2952.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Jul 2002
Last Updated on STN: 24 Jul 2002

L4 ANSWER 6 OF 14 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Bacterial small-molecule three-hybrid system based on the interaction of heterodimeric ligand-receptor interaction and use thereof for high-throughput drug screening
AB A transgenic bacterial cell comprising (a) a dimeric small mol. which comprises a first moiety known to bind a first receptor domain covalently linked to a second moiety known to bind a second receptor domain; (b) nucleotide sequences which upon transcription encode (i) a first fusion protein comprising the first receptor domain, and (ii) a second fusion protein comprising the second receptor domain; and (c) a reporter gene wherein expression of the reporter gene is conditioned on the proximity of the first fusion protein to the second fusion protein. The cell is also adapted for use in a method for identifying a mol. that binds to a known target in a bacterial cell from a pool of candidate mols., and a method for identifying an unknown target receptor to which a mol. is capable of binding in a bacterial cell. Also described are compds. and kits for carrying out the methods, in particular, the synthesis of the Mtx-SLF heterodimer. In particular embodiments, the method is exemplified by using a small mol. heterodimeric

Mtx-SLF (methotrexate-SLF(a synthetic analog of FK506)) to bridge the λcI DNA-binding domain, which is fused to FK506 receptor FKBP12 (FK506-binding protein 12), and the activation domain - αNTD (the N-terminal domain of the α-subunit of RNA polymerase), which is fused to methotrexate receptor DHFR (dihydrofolate reductase). The interaction of λcI-FKBP12 and αNTD-DHFR fusion protein leads to the transcription activation of a lacZ reporter gene, in which the λcI binding site is placed upstream of lacZ promoter. Thus, upon addition of the small mol. heterodimer Mtx-SLF, the λcI-FKBP12 and αNTD-DHFR fusion protein are dimerized, which then drives the lacZ transcription. This bacterial small mol. three-hybrid system is useful for high-throughput screening for small mol. drugs and drug-interacting protein targets.

ACCESSION NUMBER: 2005:1078092 HCAPLUS
DOCUMENT NUMBER: 143:361162
TITLE: Bacterial small-molecule three-hybrid system based on the interaction of heterodimeric ligand-receptor interaction and use thereof for high-throughput drug screening
INVENTOR(S): Althoff, Eric A.; Cornish, Virginia W.
PATENT ASSIGNEE(S): Trustees of Columbia University In the City of New York, USA
SOURCE: U.S. Pat. Appl. Publ., 28 pp., Cont.-in-part of U.S. Ser. No. 132,039.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005221402	A1	20051006	US 2005-512497	20050523
US 2003203471	A1	20031030	US 2002-132039	20020424
US 7083918	B2	20060801		
WO 2004042345	A2	20040521	WO 2003-US12612	20030424
WO 2004042345	A3	20040923		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2002-132039 A2 20020424
WO 2003-US12612 W 20030424
OTHER SOURCE(S): MARPAT 143:361162

L4 ANSWER 7 OF 14 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Directed Evolution of a Glycosynthase via Chemical Complementation
AB Recently, we reported a general assay for enzyme catalysis based on the yeast three-hybrid assay, Chemical Complementation, which is intended to expand the range of chemical reactions to which directed evolution can be applied. Here, Chemical Complementation was applied to a glycosynthase derived from a retaining glycosidase, an important class of enzymes for carbohydrate synthesis. Using the yeast three-hybrid assay, the glycosynthase activity of the E197A mutant of the Cel7B from Humicola insolens was linked to transcription of a LEU2 reporter gene, making cell growth dependent on glycosynthase activity in the absence of leucine. Then the LEU2 selection

was used to isolate the most active glycosynthase from a Glu197 saturation library, yielding an E197S Cel7B variant with a 5-fold increase in glycosynthase activity. These results not only establish Chemical Complementation as a platform for the directed evolution of glycosynthases, but also show the generality of this approach and the ease with which it can be applied to new chemical reactions.

ACCESSION NUMBER: 2004:920718 HCAPLUS
DOCUMENT NUMBER: 142:109174
TITLE: Directed Evolution of a Glycosynthase via Chemical Complementation
AUTHOR(S): Lin, Hening; Tao, Haiyan; Cornish, Virginia W.
CORPORATE SOURCE: Department of Chemistry, Columbia University, New York, NY, 10027, USA
SOURCE: Journal of the American Chemical Society (2004), 126(46), 15051-15059
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 8 OF 14 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Three hybrid assay system for isolating ligand-binding polypeptides and for isolating small mol. ligands
AB The invention provides compns. and methods for isolating ligand-binding polypeptides for a user-specified ligand, and for isolating small mol. ligands for a user-specified target polypeptide using an improved class of hybrid ligand compds. Preparation of compds., e.g a methotrexate moiety linked by a polyethylene gycol moiety to dexamethasone, is described.

ACCESSION NUMBER: 2004:182368 HCAPLUS
DOCUMENT NUMBER: 140:229401
TITLE: Three hybrid assay system for isolating ligand-binding polypeptides and for isolating small mol. ligands
INVENTOR(S): Come, Jon H.; Becker, Frank; Kley, Nikolai A.; Reichel, Christoph
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 238 pp., Cont.-in-part of U.S. Ser. No. 91,177.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 6
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
US 2004043388	A1	20040304	US 2002-234985		20020903
US 2003165873	A1	20030904	US 2002-91177		20020304
US 2004266854	A1	20041230	US 2004-820453		20040407
PRIORITY APPLN. INFO.:			US 2001-272932P	P	20010302
			US 2001-278233P	P	20010323
			US 2001-329437P	P	20011015
			US 2002-91177	A2	20020304
			US 2001-336962P	P	20011203
			WO 2002-US6677	A2	20020304
			US 2002-234985	A2	20020903
			WO 2002-US33052	A2	20021015
			US 2003-460921P	P	20030407
			US 2003-531872P	P	20031223

L4 ANSWER 9 OF 14 HCAPLUS COPYRIGHT 2006 ACS on STN
TI A Three-Hybrid Approach to Scanning the Proteome for Targets of Small Molecule Kinase Inhibitors
AB In this study, the authors explored the application of a yeast 3-hybrid (Y3H)-based compound/protein display system to scanning the proteome for targets of kinase inhibitors. Various known cyclin-dependent kinase (CDK) inhibitors, including purine and indenopyrazole analogs, were displayed in the form of methotrexate-based hybrid ligands and deployed in cDNA library or yeast cell array-based screening formats. For all inhibitors, known cell cycle CDKs as well as novel candidate CDK-like and/or CDK-unrelated kinase targets could be identified, many of which were independently confirmed using secondary enzyme assays and affinity chromatog. The Y3H system described here may prove generally useful in the discovery of candidate drug targets.

ACCESSION NUMBER: 2004:173708 HCAPLUS
DOCUMENT NUMBER: 141:362497
TITLE: A Three-Hybrid Approach to Scanning the Proteome for Targets of Small Molecule Kinase Inhibitors
AUTHOR(S): Becker, Frank; Murthi, Krishna; Smith, Chase; Come, Jon; Costa-Roldan, Nuria; Kaufmann, Christine; Hanke, Urs; Degenhart, Carsten; Baumann, Sabine; Wallner, Wolfgang; Huber, Andrea; Dedier, Severine; Dill, Simone; Kinsman, David; Hediger, Mark; Bockovich, Nicholas; Meier-Ewert, Sebastian; Kluge, Arthur F.; Kley, Nikolai
CORPORATE SOURCE: GPC Biotech AG, Planegg/Martinsried, 82152, Germany
SOURCE: Chemistry & Biology (2004), 11(2), 211-223
CODEN: CBOLE2; ISSN: 1074-5521
PUBLISHER: Cell Press
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 10 OF 14 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Bacterial small-mol. three-hybrid system comprising dimeric Mtx-SLF ligand that bridges λcI and NTD fusion proteins for detecting protein-small molecule interactions
AB The present invention provides a transgenic bacterial cell comprising (a) a dimeric small mol. which comprises a first moiety known to bind a first receptor domain covalently linked to a second moiety known to bind a second receptor domain; (b) nucleotide sequences which upon transcription encode (i) a first fusion protein comprising the first receptor domain, and (ii) a second fusion protein comprising the second receptor domain; and (c) a reporter gene wherein expression of the reporter gene is conditioned on the proximity of the first fusion protein to the second fusion protein. The cell is also adapted for use in a method for identifying a mol. that binds to a known target in a bacterial cell from a pool of candidate mols., and a method for identifying an unknown target receptor to which a mol. is capable of binding in a bacterial cell. Also described are compds. and kits for carrying out the methods. The examples describe the synthetic preparation of a heterodimer of methotrexate and a synthetic analog of FK507 (SLF), referred to as Mtx-SLF. Mtx-SLF was used to dimerize a λcI-FK506 binding protein 12 protein chimera and an αNTD-dihydrofolate reductase protein chimera.

ACCESSION NUMBER: 2003:855546 HCAPLUS
DOCUMENT NUMBER: 139:346749
TITLE: Bacterial small-mol. three-hybrid system comprising dimeric Mtx-SLF ligand that bridges λcI and NTD fusion proteins for detecting protein-small molecule interactions

INVENTOR(S): Althoff, Eric A.; Cornish, Virginia W.
PATENT ASSIGNEE(S): The Trustees of Columbia University In the City of New York, USA
SOURCE: U.S. Pat. Appl. Publ., 28 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003203471	A1	20031030	US 2002-132039	20020424
US 7083918	B2	20060801		
WO 2004042345	A2	20040521	WO 2003-US12612	20030424
WO 2004042345	A3	20040923		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2003299463	A1	20040607	AU 2003-299463	20030424
US 2005221402	A1	20051006	US 2005-512497	20050523
PRIORITY APPLN. INFO.:			US 2002-132039	A2 20020424
			WO 2003-US12612	W 20030424

OTHER SOURCE(S): MARPAT 139:346749

L4 ANSWER 11 OF 14 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Receptor-dependence of the transcription read-out in a small-molecule three-hybrid system
AB Small-mol. three-hybrid systems show promise as an in vivo alternative to affinity chromatog. for detecting small-mol. - protein interactions. While several three-hybrid systems have been reported, little has been done to characterize these systems and, in particular, to test the assumption that the protein - small-mol. interaction can be varied without disrupting the transcription read-out. Recently we reported a dexamethasone - methotrexate chemical inducer of dimerization (CID) for use in the yeast three-hybrid system, based on the well-studied ligand - receptor pairs dexamethasone (Dex) - glucocorticoid receptor (GR) and methotrexate (Mtx) - dihydrofolate reductase (DHFR). Here we describe our first efforts to characterize this system, by focusing on a comparison of the activity of a bacterial and a mammalian DHFR as a test case of the influence of the ligand - receptor pair on the transcription read-out. By using a lacZ reporter gene, the activity of several GR and DHFR protein chimeras with different orientations and linker sequences and Dex - Mtx CIDs with different chemical linkers have been compared. In addition, Western analyses and in vivo biochem. assays have been carried out to confirm the integrity of the GR and DHFR protein chimeras. The transcription read-out is found to be much more sensitive to the structure of the protein chimeras than the CID. The most surprising result is that the levels of transcription activation are consistently higher with the bacterial than the mammalian DHFR, despite the fact that both proteins bind Mtx with an inhibition constant (K1) in the low pM range. These results set the stage for understanding three -hybrid systems at the biochem. level so that they can be used to detect ligand - receptor pairs with a range of structures and dissociation consts.

ACCESSION NUMBER: 2002:694644 HCAPLUS
DOCUMENT NUMBER: 138:86027
TITLE: Receptor-dependence of the transcription read-out in a small-molecule three-hybrid system
AUTHOR(S): Abida, Wassim M.; Carter, Brian T.; Althoff, Eric A.; Lin, Hening; Cornish, Virginia W.
CORPORATE SOURCE: Department of Chemistry, Columbia University, New York, NY, 10027, USA
SOURCE: ChemBioChem (2002), 3(9), 887-895
CODEN: CBCHFX; ISSN: 1439-4227
PUBLISHER: Wiley-VCH Verlag GmbH
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 12 OF 14 HCAPLUS COPYRIGHT 2006 ACS on STN
TI A bacterial small-molecule three-hybrid system
AB The authors report the first robust bacterial RNA polymerase small mol. three-hybrid system. This system is based on the interaction between the small mol. methotrexate and a synthetic analog of FK506 (SLF) and their protein receptors, dihydrofolate reductase and FK506-binding protein 12 (FKBP12). In this assay, the binding site for the DNA-binding protein λcI is placed upstream of the promoter for a lacZ reporter gene. λCI is fused to FKBP12 and the N-terminal domain of the α-subunit of RNA polymerase (αNTD) is fused to DHFR. Thus, upon addition of the small mol. heterodimer Mtx-SLF, the λcI-FKBP12 and αNTD-DHFR fusion protein are dimerized, thus activating transcription of the lacZ gene. Synthesis of the Mtx-SLF heterodimer is described. The levels of small mol. induced transcription activation were quantified using liquid lacZ assays. The levels of transcriptional activation depend on the concentration of Mtx-SLF in the bacterial three-hybrid system. The bacterial small mol. three-hybrid system described here should provide a platform for high-throughput assays based on protein-small mol. interactions.
ACCESSION NUMBER: 2002:548931 HCAPLUS
DOCUMENT NUMBER: 137:305334
TITLE: A bacterial small-molecule three-hybrid system
AUTHOR(S): Althoff, Eric A.; Cornish, Virginia W.
CORPORATE SOURCE: Department of Chemistry, Columbia University, New York, NY, 10027, USA
SOURCE: Angewandte Chemie, International Edition (2002), 41(13), 2327-2330
CODEN: ACIEF5; ISSN: 1433-7851
PUBLISHER: Wiley-VCH Verlag GmbH
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 13 OF 14 HCAPLUS COPYRIGHT 2006 ACS on STN
TI A GAL4-based yeast three-hybrid system for the identification of small molecule-target protein interactions
AB We report the development of a yeast strain designed for assaying compound-protein interactions through activation of reporter gene expression. Activation of lacZ expression, driven by the GAL4 promoter, has been demonstrated for precedented compound-protein interactions between FK506 and FK506 binding protein 12 (FKBP12) and also between methotrexate (MTX) and dihydrofolate reductase (DHFR). Reporter gene expression was completely abrogated in a

competitive manner by the presence of excess FK506 or MTX, resp. In addition, a strain expressing a mutated DHFR clone with decreased binding affinity for MTX was not capable of activating reporter gene expression. While strain sensitivity is compound-dependent, the min. compound concentration necessary to drive reporter gene expression was 20 nM for the FK506-FKBP12 interaction. The utility of this strain as a tool for identifying unknown compound-binding proteins has been demonstrated by screening a mouse cDNA library for clones that encode proteins capable of binding MTX. Four library clones of mouse DHFR were identified after screening 5+106 clones. The screen background was low and false positives were easily identified, making this yeast system particularly amenable for use in a screening context for novel compound-protein interactions.

ACCESSION NUMBER: 2002:340741 HCAPLUS
DOCUMENT NUMBER: 138:50357
TITLE: A GAL4-based yeast three-hybrid system for the identification of small molecule-target protein interactions
AUTHOR(S): Henthorn, Debbie C.; Jaxa-Chamiec, Albert A.; Meldrum, Eric
CORPORATE SOURCE: Asthma Cell Biology, GlaxoSmithKline Medicines Research Centre, Stevenage/Hertforsdshire, SG1 2NY, UK
SOURCE: Biochemical Pharmacology (2002), 63(9), 1619-1628
CODEN: BCPCA6; ISSN: 0006-2952
PUBLISHER: Elsevier Science Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 14 OF 14 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Yeast three-hybrid system for in vivo drug screening and enzyme evolution using chemical inducers of dimerization
AB The disclosed invention relates to the evolution of enzymes in vivo, and drug screening in vivo through the use of chemical inducers of protein dimerization. The subject invention provides a compound having the formula: H1--X--B-Y--H2 wherein each of H1 and H2 may be the same or different and capable of binding to a receptor which is the same or different; wherein each of X and Y may be present or absent and if present, each may be the same or different spacer moiety; and wherein B is an enzyme cleavable moiety. This invention also provides a method of screening proteins for the ability to catalyze bond cleavage or bond formation, comprising the steps of: (a) providing a cell that expresses a pair of fusion proteins which upon dimerization change a cellular readout; (b) providing the compound of the invention which dimerizes the pair of fusion proteins, said compound comprising two portions coupled by a bond that is cleavable or formed by the protein to be screened; and (c) screening for the cellular readout, wherein a change the cellular readout indicates catalysis of bond cleavage or bond formation by the protein to be screened. However, it has not heretofore been suggested to use small mol. induced protein dimerization to screen for catalysis in vivo., and specifically, it has not been suggested to use an enzyme cleavable moiety to link two mols. to dimerize proteins. This invention provides proteins de novo with prescribed binding and catalytic properties and permits screening cDNA libraries based on biochem. function. Practically, we believe that powerful screens in combination with existing randomization techniques will make it possible to take an existing protein fold and evolve it into an enzyme with a new function generating useful catalysts for the pharmaceutical and chemical industries. Since the screen is done in vivo and in both prokaryotes and eukaryotes, the methodol. can be applied to functional genomics and drug discovery. A new chemical inducer of dimerization (CID) was recently developed in Professor Cornish's lab, which uses a heterodimer of methotrexate (MTX) and dexamethasone

(DEX) which, when placed in the yeast three-hybrid system, reconstitutes transcription of the lacZ gene. The effects of altering the structure of the DEX-MTX CID and the protein chimeras in the three-hybrid assay were investigated. It was observed that all DEX-MTX CIDs, except the DEX-MTX CID with the shortest chemical linker, showed the ability to induce β-galactosidase levels at levels 400% above strains possessing no CID. The DEX-MTX CIDs showed little or no increase in β-galactosidase levels above background levels in strains where dihydrofolate reductase (DHFR) from E. coli was replaced by DHFR from murine. The three-hybrid system did show some directional preference to the way in which the receptors where fused to the DNA binding domain and the activation domain. These studies have led to a better understanding of the factors that are important in activating transcription in the DEX-MTX yeast three-hybrid system.

ACCESSION NUMBER: 2002:31914 HCAPLUS
DOCUMENT NUMBER: 136:98820
TITLE: Yeast three-hybrid system for in vivo drug screening and enzyme evolution using chemical inducers of dimerization
INVENTOR(S): Cornish, Virginia W.
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 48 pp., Cont.-in-part of U.S. Ser. No. 490,320.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002004202	A1	20020110	US 2001-768479	20010124
US 2004106154	A1	20040603	US 2003-705644	20031110
PRIORITY APPLN. INFO.:			US 2000-490320	A2 20000124
			US 2001-768479	A3 20010124

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(FILE 'HOME' ENTERED AT 11:29:15 ON 31 AUG 2006)

FILE 'MEDLINE, BIOSIS, WPIDS, BIOTECHDS, HCAPLUS' ENTERED AT 11:33:17 ON 31 AUG 2006
L1 0 S (3-HYBRID SCREEN) AND (METHOTREXATE)
L2 30 S (Y3H OR THREE HYBRID) AND (METHOTREXATE)
L3 7 S L2 AND (FUSION PROTEIN)
L4 14 S L2 AND (REPORTER GENE)
L5 4 S L3 AND L4

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E3            0 --> CORNISH, V/AU
E4            5     CORNISHB A/AU
E5          130     CORNISHBOWDEN A/AU
E6            2     CORNISHBOWDEN A J/AU
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E11           1     CORNIST K L/AU
E12           1     CORNIST KIM LAMAR/AU

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     FILE 'MEDLINE, BIOSIS, WPIDS, BIOTECHDS, HCAPLUS' ENTERED AT 11:33:17 ON
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L2            30 S (Y3H OR THREE HYBRID) AND (METHOTREXATE)
L3             7 S L2 AND (FUSION PROTEIN)
L4            14 S L2 AND (REPORTER GENE)
L5             4 S L3 AND L4

     FILE 'SCISEARCH, MEDLINE, BIOSIS, WPIDS, DGENE, EMBASE, HCAPLUS,
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               E CORNISH, V/AU
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